

Perinatal programming of skeletal system

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Abstract: Many studies have led to the proposition that postnatal development and physiological functions of the whole organism including metabolic processes of skeletal system are established before birth. Restricted fetal growth and programmed permanent alterations in its endocrine, metabolic and skeletal systems as the physiological consequences are presented. Although the underlying mechanisms of prenatal programming of skeletal development have not yet been conclusively determined, a reduced foetal nutrient supply as a consequence of poor placental function or unbalanced maternal nutrition, and exposure to excess glucocorticoids are strongly implicated. During development, there are critical periods of exposure to suboptimal conditions. Vulnerable periods occur at different times for different tissues. Each phase of foetal development provides required conditions to subsequent development. Programming involves structural and functional effects in the skeleton that may persist for the life. Moreover, programming often has different effects in males and females.

Keywords: skeletal system, prenatal and neonatal programming, dexamethasone, alpha-ketoglutarate, pigs

Hormones and nutrients are known to operate in regulating the growth but also in maintenance stability of organism including homeostasis of bone mass in the adult. Growth is retarded in the absence or in the deficiency of some hormones, nutritional elements or after the action of inhibitors. Many diets or illness are related to the presence of clinically recognized bone diseases [1]. Diseases such as bronchopulmonary dysplasia or asthma, and corticosteroid treatment are linked with the demineralization of bones [1-3]. Other problem is preterm-born children who reach less mineralized skeleton at peak bone mass what is very important further in life for skeleton functions. There is the association between birth weight and the postnatal development not only of the whole organism [4]. Several animal models and human clinical observations were described according to the hypothesis of prenatal programming indicating that foetal growth is not only dependent on genetic factors but structural and functional properties of the central nervous system and other systems as skeleton as well on [5-7]. Inadequate nutrient supply or excess of glucocorticoids to the foetal compartments retard the growth and the development. In this idea prenatal programming relates to the permanent alteration of foetal or neonatal physiological and metabolic processes by the influence of different factors at critical times of development described as "windows" of long-lasting effects in the postnatal life and maturity [8]. Many studies showed that disturbances in programming of foetal growth and the development lead to reduced insulin sensitivity, elevated blood pressure,

endocrine or metabolic disorders, obesity, changes of behavior and osteoporosis [9, 10].

The aim of the present review is to show the importance of the influence of glucocorticoids and glutamine derivatives on skeletal system development during prenatal, neonatal and postnatal growth.

PRENATAL DEVELOPMENT OF SKELETON AND POSTNATAL BONE GROWTH

In the first trimester of human pregnancy a collagenous model of bones is formed. After eight weeks of prenatal life begins the process of bone mineralization in the centers of primary ossification. There is lack of information about the balance between synthesis and loss of bone tissue during prenatal time. Bone calcification is intensified toward the end of pregnancy [10]. About 70-80% of foetal ossification occurs in the third trimester when the process of the synthesis of the foetal skeletal collagen decreases in humans and other species as well [11]. The decline in bone markers may reflect a reduction in collagen synthesis and breakdown [10]. Pregnancy is a physiological state when calcium homeostasis is changed in order to build foetal skeleton and to maintain of normal range calcium blood plasma concentration of pregnant. In humans, calcium is necessary in a total quantity about 20-30 g for forming the skeleton at term of birth, and is provided by the maternal dietary intake, maternal extra-cellular source, and maternal skeletal system [10, 12]. The plasma foetal calcium level increases progressively from the first trimester, reaching a peak before the term. The peak accretion is at 36-38 week of gestation in humans. Other minerals are actively transported across the placenta during prenatal life. The developing foetus depends on maternal source of phosphor and magnesium [10].

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The concentration of calcium, phosphorus and magnesium is higher in the foetus than in mother. According to the highest demand for calcium for foetal bone mineralization maternal plasma osteoprotegerin (OPG) concentration also increases. It is a protection of maternal skeleton from too high losses of calcium [12, 13]. Since 1997, when the OPG/RANKL/RANK system was discovered, its central role in bone homeostasis and protection from decreased total bone density has been investigated. Normal foetal and postnatal development depends also on the glucose supply from maternal sources [7]. Moreover, maternal dietary protein feed is important for amino acids availability to the foetus, which influences foetal growth. The restriction of maternal dietary protein decreases foetal protein plasma concentration, decreases a lot of amino acids, including glutamine, proline, and arginine, which results in the foetal retardation of foetal growth. The concentration of alanine – a gluconeogenic precursor – is also decreased [14]. There is positive correlation between plasma amino acids concentration and neonatal weight [15]. There is likewise a close positive relationship between size at birth and peak bone and muscle mass. It is known that if small newborns have reduced muscle mass and strength, some metabolic functions are also changed [16, 17]. Postnatal growth relates not only to skeleton but also to muscle mass and strength; both increase during childhood, reach a peak in early adulthood and after 35-40 years in humans the decrease is observed [18]. Age related bone loss was investigated in the relation to endocrine system which comes within the programming. Growth hormone, insulin like growth factor-1 (IGF-1), hypothalamo-pituitary-adrenal axis and gonadal steroids are involved in the mechanism of the programming of bone and muscle mass. Nutritional state plays a major role in regulating their circulating concentration [19, 20]. The regulating mechanism underlying the endocrine programming, when disturbed, leads to the reduction of peak bone and muscle mass by the decrease of the mineralization and altered rate of bone loss during later life [16].

GROWTH RESTRICTION (RETARDATION) AND CATCH-UP IN THE PROGRAMMING OF SKELETAL DEVELOPMENT

Several different manipulations in the development during pregnancy or the first early neonatal period affect not only general growth, but also foetal skeletal maturity [5]. During prenatal life, the development of the foetus is dependent on the mother and the maternal environment for its nutritional needs. The influence of different factors on foetal growth results in changes in the metabolism of foetus, and may show effects long after the removal of these factors [21]. This theory of programming is based on the capacity for the adaptation, dependent on the plasticity of biologic systems of the foetus and neonates during development. These nutritional or hormonal influences change the structure of the developing systems such as the nervous and skeletal systems, and may be permanent later in life [4]. The possibility of influencing the general growth and development of the foetus occurs only during certain periods known as critical periods of time and described as “windows” of sensitivity for different factors. These “windows” were described in rats and sheep [21]. Undernutrition or malnutrition of maternal animals during a selected period of pregnancy results in growth restriction in newborns [17]. Intra-uterine growth retardation (IUGR) has also been

reported in children born of dexamethasone-treated mothers [22]. Many studies have reported changes in the endocrine parameters of the somatotrophic axis in programmed offspring with growth restriction. Indeed, reduced growth in foetus is linked with reduced IGF-1 concentration which results in growth deficit [23]. Foetal growth retardation resulted to a comparable degree with the reduction of the weight of the foeto-placental unit by 25-30%. Sensitivity of the growth plate to growth hormone was also altered during the prenatal time [5, 24].

Other authors have reported the possibility of neonatal programming of skeleton [25, 26]. When optimal nutrition is provided to newborns born with retarded growth, they may show rapid growth called catch-up what means come up, and reach the same state of the development as the controls. Different times of catch-up growth have been described in humans and a variety of animal models [17, 27]. The same studies suggest that neonatal time when catch-up occurs is more important than events *in utero*. The degree of newborn nutrient and time of catch-up growth may modulate the programming of the general development in restricted offspring [16, 25, 27]. The mechanism of neonatal programming of skeleton is dependent on the rate of catch-up growth. Further restricted feeding after the birth delayed the phenomenon of catch-up in the presence of continued and elevated ghrelin concentration. Ghrelin is an important appetite stimulator secreted by the stomach and stimulates growth hormone release [28]. These offspring further restricted during the neonatal time showed a reduced body weight and lean and fat body mass [25]. Retarded offspring with reduced body weight had increased plasma ghrelin and decreased plasma leptin. Both leptin and ghrelin are involved in the hypothalamic regulation of energy homeostasis. Lowered leptin induce hyperphagia in the postweaning time in these offspring and increased ghrelin [25].

GLUCOCORTICOID-INDUCED OSTEOPOROSIS

Glucocorticoid-induced osteoporosis in humans is dose-dependent (risk increases substantially at doses above the equivalent of 7 mg of prednisone per day), and also correlates with long-term treatment and a cumulative dose of glucocorticoids [44]. The osteoporotic effect of glucocorticoids is usually detectable after 3 months of therapy. Skeletal wasting is most rapid during the first 6 months of therapy; trabecular bone is affected to a greater degree than cortical bone. Even inhaled steroids have been shown to increase bone loss. It is not known whether there is a threshold dose of glucocorticoid below which osteopenia does not occur in human beings [2]. For several reasons, glucocorticoid-induced osteoporosis is a common complication in children and adolescents. It is generally accepted that moderate-to-high-dose glucocorticoid therapy is associated with loss of bone mass and increased risk of fracture. Childhood and adolescence are periods of high bone turnover, with very high rates of bone formation required to maintain adequate mineralization of the rapidly growing skeleton [2]. The use of glucocorticoids during adolescence prevents the patient from reaching peak bone mass; this is because glucocorticoids inhibit bone formation, and a number of pediatric disorders for which glucocorticoids are prescribed, are independently associated with osteoporosis [45-49]. The magnitude of this

problem has been demonstrated by cross-sectional studies, which suggest that the majority of subjects receiving long-term glucocorticoid therapy have low bone mineral density [20, 27, 49-53]. Thus, glucocorticoid-induced osteoporosis is an important clinical problem which commands the physician's attention to both prevention and treatment. The action of glucocorticoids was described, but there must be emphasized that synthetic glucocorticoids act in the same way as natural glucocorticoids [51]. The regulatory mechanism of prenatal programming dependent on nutrient availability or reduction and hormones (glucocorticoids) influence the risk of osteoporosis in childhood and in the adult.

The hypothesis that plasticity and adaptation in prenatal and neonatal programming fits the response of the involvement of steroid hormones into glucocorticoid-induced osteoporosis has been investigated in recent last years [73]. According to other reports, maternal stress or prenatal exposure to glucocorticoids induce growth retardation *in utero* and result in the reduction of birth weight. We therefore tested this model [73]. Foetal exposure to an excess of glucocorticoids mimics outcomes similar to those observed in other maternal nutritional models of programming which suggested functioning of the same mechanisms in these models [4, 5, 17, 27, 74]. Glucocorticoids are described as a key with a pivotal role in foetal programming [6]. In our studies we transiently inhibited longitudinal bone growth in piglets by administering synthetic glucocorticoid in the form of dexamethasone to sows during the last 3 weeks of pregnancy and/or through 2 weeks of neonatal life [73]. We assessed mechanical and geometric bone properties, bone mineral density (BMD), and bone mineral content (BMC) to determine whether the development had been delayed by the previous growth inhibition of dexamethasone [73]. There is a close association between bone structure, mineral content and endurance [75]. Our study showed the catabolic action of dexamethasone on bone development and bone mineral density. Newborn piglets treated with dexamethasone during their prenatal and neonatal life had shortened limb bones [73]. Volumetric and mineral bone density and mineral bone content were also lowered under the influence of dexamethasone. The geometric parameters indicated that the skeletal system did not mature [76]. Dexamethasone caused the bones to be thinner and weaker than these in the control group [73, 76-78]. Dexamethasone administered during the perinatal period detrimentally influences the skeletal system and its development and the processes of its mineralization, together with a negative effect on the whole body mass [76].

Our earlier studies showed that the enteral administration of the glutamine derivative alpha-ketoglutarate (AKG) to piglets and turkeys in postnatal life increased the entire mechanical and geometrical parameters of the examined bones, as well as mineralization process [79-83]. Enteral administration of AKG also reduces the gastrectomy-evoked bone loss in female rats [84]. In particular, gastrectomy causing bone loss clearly shows the great impact of gastrointestinal tract on bone homeostasis. This seems to indicate that there is an axis connecting the gut and bones. The digestive tract plays an important role in the maintenance of density and mass of bones [84-88]. However, our experiment proved that sows administered with AKG during the last 24 day of pregnancy delivered heavier piglets, but their humeri were less mineralized than in the control group; this was additionally confirmed by the ultimate strength,

maximum elastic strength, BMD, and BMC values. Prenatal administration of AKG increased the length and weight of the examined bones [78, 89]. These prenatal effects on the processes of bone mineralization are opposite to the effects observed during development in the postnatal period [78-83]. It is very important to emphasize that although the limb bones of newborns under the prenatal influence of alpha-ketoglutarate were less mineralized, and were more mature in the geometry than those of the controls. When AKG was further administered for 2 weeks after the birth of the piglets, the bones were more stable than those in the control group [90].

On the other hand, these negative effects of dexamethasone on fetal development of skeletal system were significantly reduced when simultaneous administration of AKG was performed. These effects were confirmed by a higher mechanical endurance of the bones, and resulted in improved bone geometric parameters and mineralization. The obtained results suggest that the administration of alpha-ketoglutarate during pregnancy may be beneficial for diminishing or eliminating disadvantages of skeletal development of fetuses resulting from glucocorticoids [78, 91].

Nowadays, we performed our studies to check the influence of dexamethasone administering through the last 45 days of prenatal life. Results indicate that prenatal exposure of piglets to excess of glucocorticoid such as dexamethasone during this time of pregnancy in sows disturbed metabolic processes in skeletal system and influenced the processes involved in programming of skeletal development which may persist and appear later in life. Effects of dexamethasone prenatal exposure are sex-dependent and induce stronger negative consequences in males than in females [92].

THE GENERAL ROLE OF GLUTAMINE

Glutamine and glutamate with proline, histidine, arginine and ornithine compose the "glutamate family" of amino acids. In mammalian cells, glutamine is a key metabolic between carbon metabolism of carbohydrates and proteins and plays an important role in the growth [54]. It improves nitrogen balance and preserves the concentration of glutamine in skeletal muscle [55]. When the plasma glutamine is insufficient, glutamine release occurs from skeletal muscle. Glutamate is metabolized to glutamine in a reaction which requires ammonia, ATP and magnesium. Glutamine participates in the formation of purine and pyrimidine nucleotides, glucosamine, nicotinamide adenine dinucleotide and glutathione [56]. It also participates in protein synthesis, energy production and, if necessary, the production of glucose and glycogen. Glutamine can serve as the energy in enterocytes and lymphocytes. Glutamine is considered as an immunonutrient, and supplemental glutamine is used in medical foods for such stress situations as trauma, cancer, infections and burns [56].

THE ROLE OF GLUTAMINE AND NERVOUS SYSTEM IN BONE FORMATION

Bone homeostasis keeping the balancing mechanism at least in part involves the endocrine control by estrogen and parathyroid hormone as well as the paracrine (auto-

crine) control by interleukin, insulin-like growth factor, fibroblast growth factor, transforming growth factor- β , and bone morphogenetic protein. There is a possibility that glutamate may be also one of the endogenous paracrine (autocrine) factors used for intercellular communications in bone cells including osteoblasts, osteoclasts, and osteocytes [66, 67].

The mechanisms controlling bone formation and resorption are still incompletely understood. Glutamate receptors, transporters and proteins that regulate glutamate release, are all expressed in osteoblasts, osteocytes and osteoclasts, and influence the activities of these cells [68-71]. Bone cells (osteoblasts, osteoclasts, and osteocytes) could express all molecular machineries required for glutamate signaling in the CNS. Glutamate signaling could be ignited through the exocytotic release from vesicles expressing vesicular glutamate transporters for condensation, followed by input via ionotropic glutamate receptors and/or metabotropic glutamate receptors and subsequent termination by glutamate transporters in bone cells as seen at glutamatergic synapses in the brain. Accordingly, glutamate could play a dual pivotal role in mechanisms associated with maintenance of homeostasis as an excitatory neurotransmitter toward neurocrine processes in the CNS and as a trophic factor toward autocrine and/or paracrine processes in bone [72].

Glutamate may be involved with mechanic signal transduction in bone. This idea has been supported by a wealth of evidence revealing that glutamate receptors are expressed and functional in bone cells and their activation can modulate bone cell phenotype. Glutamate receptor activation affects both osteoblast and osteoclast phenotypes revealing a potential for therapeutic manipulation of glutamate signaling to enhance bone formation. Glutamate transporters contribute to this system by regulating extracellular glutamate concentrations and acting as glutamate-gated ion channels. Artificial regulation of glutamate receptors or transporters may be used to increase the bone forming capacity of osteoblasts [71].

Glutamate is the mediator of excitatory signals in the mammalian central nervous system and is probably involved in most aspects of normal brain function including cognition, memory and learning [57, 58]. Glutamate also plays major roles in the development of the central nervous system, including synapse induction and cell differentiation. Most neurons have glutamate receptors in their plasma membranes [59, 60]. Further, glutamate plays a signaling role also in peripheral organs and tissues as well as in endocrine cells [61]. Glutamate exerts its signaling role by acting on glutamate receptors located on the surface of the cells [56].

Glutamatergic signaling is important in the regulation of nervous system development [57, 58]. The importance of glutamatergic signal transduction is the fact that the properties of the glutamate receptors change during development. There are dynamic changes in both the expression and the subunit composition of the NMDA receptors, ionotropic receptor for glutamate (N-methyl D-aspartate), and metabotropic receptors [62, 63]. The sensitivity of the developing brain to glutamatergic overstimulation (excitotoxicity) and hypoxia-ischemia is high and varies with the developmental stage e.g. the rat hippocampus is most sensitive at around the second postnatal week [64]. Because the fetal blood-brain barrier is incomplete the placental glutamate uptake is important for normal brain development [65].

The control role of the central nervous system (CNS) in bone development is known through the inhibition of bone formation after intracerebroventricular administration of leptin [29]. Neonates are exposed to high levels of leptin during the perinatal and suckling periods between days 3-14 of life often called the adrenal hypo-responsive period [30, 31]. Leptin, a hormone produced primarily in mature adipose tissue, but also in placenta and mammary epithelial cells, is positively correlated with fat mass and the percentage body fat. In human neonates, bone metabolism is linked to leptin whereby, as leptin increases with gestation, the formation of bone collagen matrix decreases [32]. The increase in leptin levels with gestational age coincides with adipose tissue development, which suggests that leptin may play a role in the regulation of bone metabolism in the developing skeleton. Indeed, high levels of gene expression for leptin and the leptin receptor have been found in the cartilage/bone of the mouse fetus [33].

Leptin receptor is localized in the ventral hypothalamus for the control of bone formation. They are also strictly connected with the release of catecholamine from sympathetic nervous system (SNS) and adrenergic receptors [34]. β_2 -adrenoreceptors are present on osteoblasts [29]. No other adrenergic receptor subtype was detected [34]. Indeed, leptin acts also specifically through β_3 -adrenoreceptors on adipocytes. The administration of anyone β_3 -adrenergic agonist reduced plasma leptin level in mice [35]. Thus, the SNS inhibits plasma leptin secretion from adipocytes in humans and animals, but β_3 -adrenoreceptors are more numerous in adipose tissue in rats than in humans. There is the loop between the adipose tissue and the hypothalamo-pituitary-adrenal axis (HPA) by which leptin stimulates sympathetic nervous system and, in turn, inhibits leptin expression from adipose tissue [35, 36]. The role of leptin in the response of HPA axis is still not fully defined, but the study on developing rats showed that it is mediated by the increase of glucocorticoid receptors in the hippocampus and hypothalamus [32, 35, 37, 38]. Because glucocorticoids elevate leptin concentration, it is possible that leptin may be involved in the mechanisms behind the observations of elevated body fat mass and reduced growth and bone mass caused by treatment with dexamethasone [39]. Interestingly, earlier studies showed that the feeding components of maternal concern are critical for maintaining low adrenal sensitivity to functional relationship between leptin and glucocorticoid existing in the regulation of the neonatal HPA axis [40]. During the neonatal period, a unique pattern of glucocorticoid receptors (GR) concentrations exists in the brain and pituitary as a base of a negative feedback action on the HPA axis controlling glucocorticoid overproduction [37, 41, 42]. Efficient glucocorticoid negative feedback on the HPA axis is critical, because low stable glucocorticoid levels are optimal for neuronal development in glucocorticoid-sensitive brain regions [43]. The effect of leptin on brain has important physiological implications for the maturation of hippocampal functions during the period of intensive neurogenesis and synaptogenesis in the developing rat, and leptin might provide a critical factor mediating the maternal and environmental effects on neonatal physiology. Correlation between leptin circulating and glucocorticoids in neonates is very important for further understanding of the relationships among infant nutrition, growth, and body composition.

IN CONCLUSION

We demonstrated that prenatal chronic exposure of pregnant sows to dexamethasone leads to the changes of foetus growth patterns and bone mass. Fetal exposure to glucocorticoids at higher than physiological levels occurs during maternal stress and iatrogenic glucocorticoid administration. Mechanism leading to programming of bone development, after a short prenatal dexamethasone exposure, is linked with the alteration in endocrine system [92, 93, 94]. Moreover, to understand the mechanisms of the improving role of AKG during glucocorticoid treatment need further investigation. Our studies for the first time present the results indicating that maternal administration of AKG simultaneously with dexamethasone markedly increases bone mineral density, mechanical and geometric properties of pig fetuses.

Moreover, the early life environmental, nutritional and hormonal factors may influence further programming of the skeletal system and induce disfunctions and increase risk of diseases, in part by inducing persistent alterations in major endocrine and neuroendocrine axes, such as the somatotrophic axis (growth hormone and the insulin-like growth factors), the hypothalamo-pituitary-adrenal axis, the adipoinular axis including leptin and the sympathetic nervous system. Indeed, the influence of sex hormones on the skeleton development needs more clarifications especially in relation to cortisol and placental enzymes [95].

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